Review Article

THE APPEARANCE OF THE THYMUS AND THE INTEGRATED EVOLUTION OF ADAPTIVE IMMUNE AND NEUROENDOCRINE SYSTEMS

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ABSTRACT

The immune system may be considered as a sensory organ able to respond to different kinds of danger signals that are not detected by nervous cells. The immune response is not autonomous but also regulated by the central and peripheral nervous system, as well as by neuropeptides, vitamin D and neuroendocrine axes such as the corticotrope, somatotrope, thyrotrope and gonadotrope axes. During evolution, the thymus emerged concomitantly with recombinase-dependent adaptive immunity as an 'immune brain' or a 'master class' highly specialized in the orchestration of central immunological self-tolerance. This was an absolute requirement for survival of species because of the high risk of autotoxicity inherent to the stochastic generation of extreme diversity characterizing this novel adaptive type of immune defenses against non-self. The thymus now appears to be an obligatory intersection for the integrated evolution of the major systems of cell-to-cell signalling, the nervous, endocrine and immune systems. The presentation of many self-peptides by thymic major histocompatibility complex (MHC) proteins is controlled by the autoimmune regulator (AIRE) gene/protein and is responsible for the clonal deletion of self-reactive T cells. In the same time, by still unexplained mechanisms, MHC presentation of the same self-peptides in the thymus promotes the generation of self-specific FOXP3+ CD4+CD25+ natural regulatory T cells (nTreg) that are able to inhibit in periphery self-reactive CD4+ and CD8+ T cells having escaped the thymus censorship. Moreover, a thymus dysfunction is more and more established as the primary event driving the development of organ-specific autoimmunity, which is the tribute paid, mainly by mankind, for the preservation of self against non-self. Our novel knowledge about thymus physiology and physiopathology already serves as the basis for the development of various innovative and efficient immunomodulating strategies in pharmacology.

Key words: Thymus, self-tolerance, autoimmunity, evolution

REVIEW

Galen (129 – 210 or 216 AD) first described an organ located behind the sternum that he named 'thymus' because of its close resemblance with a leaf of the thyme plant. For Galen, the thymus was the 'seat of soul, eagerness, and fortitude', and this old misconception most probably explains why some terms like '*troubles thymiques*' are still used in the French medical language to designate mood disorders such as those observed in unipolar and bipolar depressive diseases. Jacobus Berengarius Carpensis (1460-1530) then provided the first complete anatomical description of the thymus in his work entitled '*Anatomia Carpi. Isagoge breves perlucide ac uberime, in anatomiam humani corporis*'.

For a very long time, the thymus was considered as a useless vestigial organ that had become redundant during both phylogeny and human ontogeny after puberty. It is only in the early 1900s' that the first 'thymologist' J. August Hammar initiated in Sweden biomedical research focusing on this organ (1). His pioneering work was followed by numerous studies that have highlighted the important neuroendocrine regulation of the thymus, in particular by the hypothalamo-hypophysial axis, thyroid hormones, adrenal and sex steroids. For a long time, the thymus was considered as a gland and an intrinsic component of the endocrine system until the elucidation of its fundamental role in immunity (2). Starting from 1959, one can distinguish the following milestones leading to our current knowledge in thymus physiology:

- Role of the thymus in mouse leukaemia and in T-cell development (3, 4).
- Developmental biology of and self-recognition by differentiating T cells in the thymus (5, 6).
- Promiscuous expression by thymus epithelium of genes encoding neuroendocrine-related and peripheral tissue-restricted antigens (7-11).

- Identification of the autoimmune regulatory (Aire) gene/protein as a transcription-like factor controlling promiscuous gene expression in thymus epithelium (12, 13).
- Intrathymic selection of self-antigen specific natural regulatory T cells (nTreg) (14-16).
- Embryology of the thymus and deciphering of the lympho-stromal interactions required for T-cell differentiation in the primary lymphoid organ (17-19).

In all living species, the neuroendocrine and innate immune systems have evolved in parallel and still coexist without any apparent problem (Fig. 1). Indeed, Toll-like receptors (TLR) that are the most important mediators of innate immunity do not have the capacity of reaction against normal self. Some anticipatory immune responses already existed in jawless vertebrates (agnathans), and were mediated by diverse variable lymphocyte receptors (VLR), with 4-12 leucine-rich repeat modules assembled by a gene conversion process. Some 450-500 million years ago, the emergence of transposon-like recombination activating genes *RAG1* and *RAG2* in jawed vertebrates (gnathostomes) promoted the development of adaptive immunity (20-22). The appearance of *RAG1* and *RAG2* in the genome of jawed vertebrates (most putatively via horizontal transmission), and the subsequent appearance of the combinatorial immune system, has sometimes been assimilated to the immunology's 'Big Bang'. Gene recombination in somatic lymphoid cells is responsible for the random generation of diverse immune receptors for antigens, B-cell- ($\pm 5 \times 10^{13}$ BCR combinations) and T-cell receptors ($\pm 10^{18}$ TCR combinations). Because of its inherent risk of

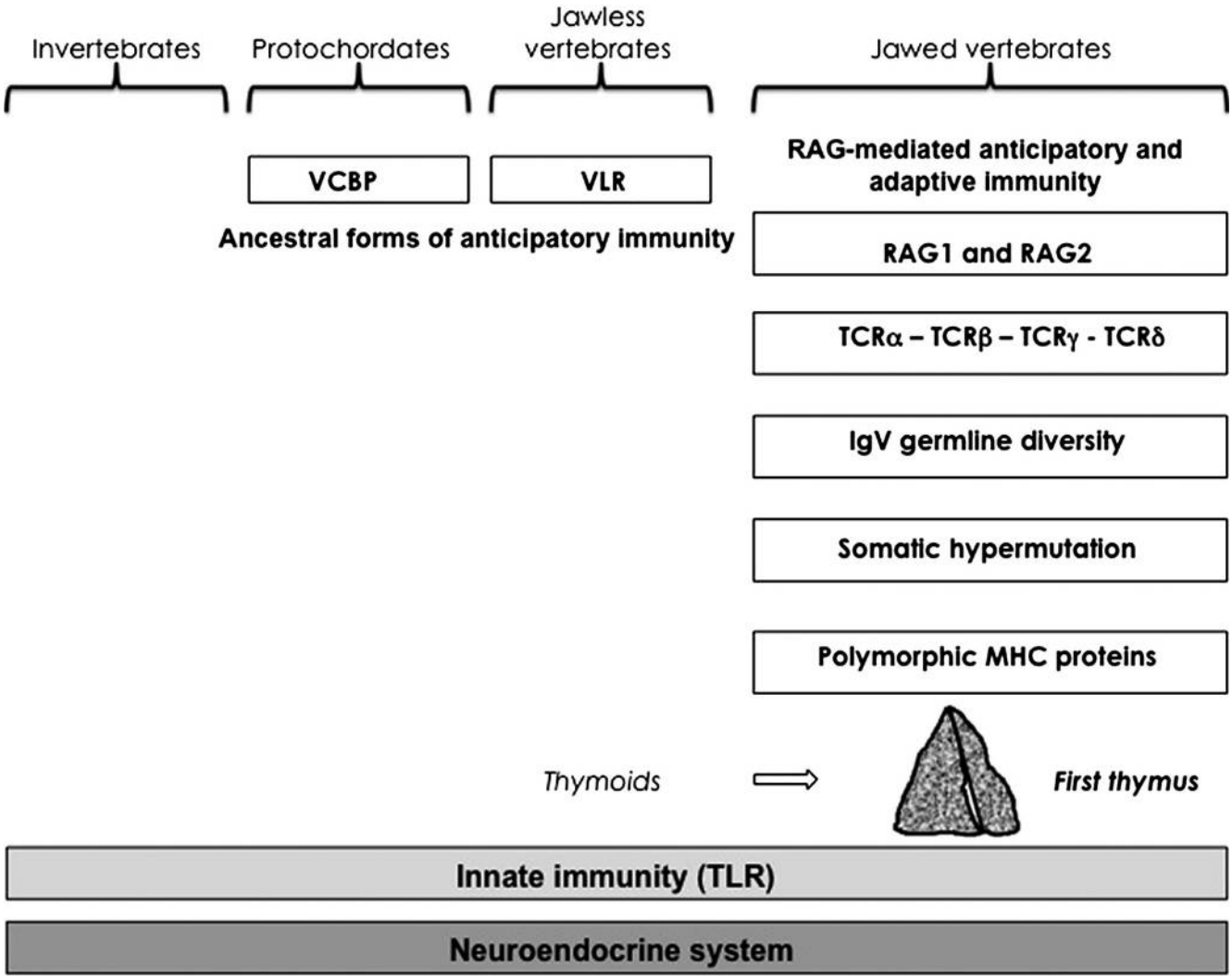

Figure 1: *Integrated evolution of the neuroendocrine system, innate and RAG-dependent immunity.*
Essential components of the neuroendocrine system have been established long ago and did not display important variation during evolution besides gene duplication or differential RNA splicing. The appearance of RAG-dependent adaptive immunity in jawed vertebrates was associated with a high risk of autotoxicity directed against the neuroendocrine system. Of note, from ancestor lamprey thymoids, the first unique thymus emerged quite concomitantly in jawed fishes, and the intrathymic presentation of neuroendocrine-related genes may be viewed a posteriori as a very efficient and economic way in instructing the adaptive T-cell system to tolerate neuroendocrine antigens as early as during intrathymic T-cell development and differentiation.
VCBP: variable-region-containing chitin-binding protein; VLR: variable lymphocyte receptor; TCR: T-cell receptor.

autotoxicity, the emergence of this sophisticated type of immune response exerted an evolutive pressure so powerful that, in concordance with Paul Ehrlich's concept and prediction of *'horror autotoxicus'*, novel structures and mechanisms appeared with a specific function in the setting-up of immunological self-tolerance. Of note, the first thymus appeared in cartilaginous-jawed fishes but was preceded by thymus-like lympho-epithelial structures in the gill baskets of lamprey larvae as very recently demonstrated (23). These structures named '*thymoids*' express the gene encoding forkhead box N4 (FOXN4), the orthologue of FOXN1. FOXN1 is the transcription factor specific for the differentiation of thymus epithelium in jawed vertebrates, and *Foxn1* mutation is responsible for the nude phenotype in mouse. Therefore, FOXN1 stands at a crucial place in the development of thymus epithelium that is an absolute requirement for T-cell differentiation. Moreover, the same study has provided strong evidence for a functional analogy between VLR assembly in these thymoids and TCR recombination in the thymus. This important discovery opens the question about the potential existence of autoimmune-like responses in jawless vertebrates.

Two essential and closely associated mechanisms are responsible for ensuring the thymus-dependent central arm of self-tolerance: 1) negative selection of self-reactive T cells that are stochastically generated by recombinase-dependent generation of TCR diversity in the thymus (*recessive* tolerance), and 2) positive selection of self-specific nTreg, which are able to inactivate in periphery self-reactive T cells having escaped thymic negative selection (*dominant* tolerance). Today, the major unresolved question is to understand the precise mechanisms by which the same associations of self-antigens and thymic major histocompatibility complex (MHC) proteins are able to mediate both dominant and recessive self-tolerance (reviewed and discussed in 24).

Another question has long concerned the nature of self that is presented in the thymus to differentiating T cells during foetal life. Since its formulation some 75 years ago, 'self' has been a seminal word coined in immunological language as a fecund metaphor with some equivocal correlations to philosophy and neurocognitive sciences. For unknown reasons, there was no serious attempt to elucidate the precise identity of self before a series of consecutive studies in the late 1980s and in the 1990s (7, 25-30). Our personal contribution in this field was to define the biochemical nature of the neuroendocrine self. First, thymic neuroendocrine self-antigens usually correspond to peptide sequences that have been mostly conserved throughout evolution of their related protein family. Second, a hierarchy characterizes their expression profile in the thymus as one dominant member synthesized in thymus epithelium represents its related neuroendocrine family in front of differentiating T lymphocytes (*i.e.* oxytocin for the neurohypophysial family, neurokinin A for tachykinins, neurotensin for neuromedins, corticostatin for somatostatins, and insulin-like growth factor 2 [IGF-2] for the insulin family). This hierarchical pattern is meaningful because the strength of immunological tolerance to a protein is proportional to its intrathymic concentration (31). Third, following Aire-regulated gene transcription, thymic neuroendocrine precursors are not processed according to the classic model of neurosecretion but undergo an antigen processing for presentation by, or in association with, thymic MHC proteins. Finally, for some of them, their transcription in the thymus precedes their eutopic expression in neuroendocrine glands (32).

This hierarchy in the organization of the thymic repertoire of neuroendocrine self-antigens is also significant from an evolutionary point of view. Since many major physiological functions had been established before the emergence of the anticipatory adaptive immune response in jawless fishes, they had to be protected from the risk of autoimmunity inherent to this type of immune lottery. Oxytocin is a hypothalamic neuropeptide that is closely implicated at different steps of the reproductive process, starting from social affiliation and bonding to control of parturition and lactation. Thus, this neuropeptide is fundamental for preservation of animal and human species. Through its dominant expression in thymus epithelium, oxytocin is more tolerated than its hypothalamo-neurohypophysial homologue vasopressin, which essentially controls water homeostasis. Interestingly, rare cases of autoimmune hypothalamitis with vasopressin deficiency and diabetes insipidus have been repeatedly observed whereas any autoimmunity towards hypothalamic oxytocinergic neurons has never been reported. A similar reasoning may be applied to the members of the insulin family, IGF-2, IGF-1 and insulin itself. There is no report of autoimmunity against IGF-2, the dominant thymic self-peptide of the insulin family during foetal life, whereas insulin is the primary autoantigen of type 1 diabetes. Because of their close homology, thymic neuroendocrine self-antigens promote immunological cross-tolerance to their whole family, and tolerance to insulin was shown to be weaker in *Igf2*$^{-/-}$ mice than in wild-type mice (33).

As already theorized by Burnet, the pathogenesis of autoimmune diseases may first depend on a failure of self-tolerance and the development of 'forbidden' self-reactive immune clones (34). The progressive increase in immune complexity during evolution is associated with a higher incidence of self-tolerance failures, most of them occurring in the human species. There is more and more evidence that a dysfunction in the mechanisms responsible for thymus-dependent dominant and recessive self-tolerance is playing a primary role in the development of the autoimmune response toward many organs. Thymus transplantation from non-obese diabetic (NOD) mice, an animal model of type 1 diabetes, was shown to induce diabetes in normal recipients (35). *Igf2* transcription is deficient in the thymus of diabetes-prone Bio-Breeding (DPBB) rats, another animal model of type 1 diabetes, and such defect might contribute to both the absence of tolerance to β cells and the usual lymphopenia (including RT6+ Treg) observed in these animals (36). Mice with thymus-restricted insulin defect develop strong proinsulin-specific T-cell reactivity (37). Loss-of-function *Aire* single mutations are responsible for a very rare autosomal recessive disease named autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) or autoimmune polyglandular syndrome type 1 (APS-1). Depending on their genetic background, *Aire*$^{-/-}$ mice exhibit several signs of peripheral autoimmunity, which are associated with a significant decrease in the level of intrathymic neuroendocrine gene transcription, including those encoding oxytocin, insulin and IGF-2 (38, 39). Of note with regard to autoimmune thyroiditis, which is the most frequent autoimmune disease, all major thyroid-related antigens (thyroperoxydase, thyroglobulin and

thyrotropin receptor [TSHR]) are also transcribed by thymic epithelium in normal conditions (29, 40). Thymic hyperplasia is commonly observed in Graves' disease (1, 41), and it was recently shown that homozygotes for an SNP allele predisposing to Graves' disease have significantly lower intrathymic *TSHR* transcripts than carriers of the protective allele (42). Another recent credit to a defective central tolerance in organ-specific autoimmune disease was provided by a very elegant study showing the central role played by a defect of intrathymic α-myosin expression in autoimmune myocarditis in mice and humans (43). Our current in-depth knowledge in thymus physiology and physiopathology should translate very soon into the design of innovative tolerogenic and regulatory strategies aimed at restoring central self-tolerance that is defective in autoimmunity, the heavy price paid by so many patients for preserving human self against non-self (44, 45).

Immunoneuroendocrinology was recognized as a scientific field early in the 20th century, soon after Paul Ehrlich identified immunology as a specific domain of scientific investigation. By the 1930s, Hans Selye introduced the concept of stress-induced and adrenal cortex-mediated thymus involution and secondary immunosuppression. The dissection of the intricate cellular and molecular interactions between the major systems of cell-to-cell signalling – the neural, endocrine, and immune systems – was relaunched in the 1980s but this scientific domain has received only gradual acceptance by the scientific community. Endocrinologists did not hesitate to widely open the door to this new field and provided the first robust experimental arguments for its fundamental relevance to physiology. Immunoneuroendocrinology has been expanded exponentially and the immunological self-tolerance of neuroendocrine proteins is now recognized as an obvious necessity for preserving general homeostasis of living organisms. Indeed, all hormones and neuropeptides exert an important control upon the immune and inflammatory responses through binding to and activation of neuroendocrine receptors expressed by immunocompetent cells (reviewed in several chapters of 46, 47). If self-tolerance to neuroendocrine ligands and receptors were not firmly installed, then the risk of developing autoimmune phenomena would be extremely high and species survival would be severely compromised.

Aging of the immune system (immunosenescence) is characterized by a higher susceptibility to infections, an increase in the incidence of cancer, as well as a decrease in response to vaccines. Although thymopoiesis (generation of naïve T cells) is maintained until late in life, thymus adipose involution has been long considered as 'the' hallmark of immunosenescence. Thymic involution is associated with a marked decrease in the generation of diverse T cells (in particular naïve CD4+ T cells), an expansion of memory CD8+ T cells, and a diminished influence of thymus-dependent central self-tolerance. Involution of the thymus after hypophysectomy was one of the first evidences for the control of the immune system by a neuroendocrine structure (48). Numerous studies have unambiguously demonstrated that the antehypophysial growth hormone (GH) is able to reverse the age-dependent involution of the thymus (49-51). The intrathymic proliferation of T-cell precursors and thymic output of naïve T cells are significantly decreased in adults with GH deficiency and GH replacement restores these two parameters (52). Today, the restoration of thymus function appears as an important objective in the elderly, as well as in patients suffering with AIDS or several hematological diseases (53, 54). It can now be anticipated that GH, IGF-1, GH secretagogues (such as ghrelin), GH and ghrelin receptor agonists, as well as other thymus-specific growth factors will be used in the near future for regenerating thymopoiesis and thymus tolerogenic function as well as, secondarily, several immune functions including responses to vaccines in aged and other immunocompromised patients.

In conclusion, as discussed in this short overview, a novel era is now widely open for objective clinical investigation of thymus function in a variety of immune and infectious diseases. Moreover, the pharmacological manipulation of both thymus-dependent thymopoietic and tolerogenic function will provide the scientific community with innovative strategies for the treatment of a large number of immune-mediated disorders.

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